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MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

Tamara C. Burke

A Project Presented in Partial Fulfillment
of the Requirements for the Degree
Masters of Science
in Environmental Biology

REGIS UNIVERSITY
May, 2018

MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

Tamara C. Burke

has been approved

May, 2018

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Table of Contents

CHAPTER 1. LITERATURE REVIEW: INVESTIGATING THE INFLUENCE OF ABIOTIC VARIABILITY ON ESCHERICHIA COLI POPULATION DYNAMICS IN THREE DENVER, CO STREAMS	6
References	14
CHAPTER 2. GRANT PROPOSAL: INVESTIGATING THE INFLUENCE OF ABIOTIC VARIABILITY ON ESCHERICHIA COLI POPULATION DYNAMICS IN THREE DENVER, CO STREAMS	16
Abstract	16
Project Description.....	16
Background/Rationale/Significance	16
Methods.....	20
Field Collection.....	20
Lab Analysis	21
Statistical Analysis.....	22
Work Plan	22
Relation to Course work/Career goals	23
References	24

CHAPTER 3. JOURNAL MANUSCRIPT: INVESTIGATING THE INFLUENCE OF ABIOTIC VARIABILITY ON ESCHERICHIA COLI POPULATION DYNAMICS IN THREE DENVER, CO STREAMS	26
Abstract	26
Introduction	28
Methods	32
Study Area	32
Field Collection	33
Laboratory Analysis	9
Statistical Analysis	9
Results	10
Study Site and Water Quality Characteristics	10
Spatiotemporal drivers of variation in E. coli concentrations	11
E. coli Decay Rate Spatiotemporal Relationships	12
Discussion	17
References	22
CHAPTER 4. ENVIRONMENTAL STAKEHOLDER ANALYSIS: A CALL TO BAN RIVERFRONT DEVELOPMENT IN DENVER, CO TO IMPORVE WATER QUALITY	25
References:	30

FIGURE AND TABLE LIST

CHAPTER 1, LIST OF FIGURES

1. Abiotic Influence diagram on *E. Coli* Concentrations.....8

CHAPTER 2, LIST OF FIGURES

2. Abiotic Influence diagram on *E. Coli* Concentrations.....16

CHAPTER 3, LIST OF TABLES

1. Abiotic variable data summary37
2. Univariate Regression Results37
3. Best Models39

CHAPTER 3, LIST OF FIGURES

3. Sampling Map.....31
4. IDEXX Tray for *E. coli* Enumeration.....33
5. Surface and Bottom *E. coli* Concentration Plot37
6. Surface and Bottom *E. coli* Decay Rate Plot38
7. Regression relationship between *E. coli* decay rate and specific conductivity.....39
8. Regression relationship between *E. coli* decay rate and pH.....39

CHAPTER 1. LITERATURE REVIEW: INVESTIGATING THE INFLUENCE OF ABIOTIC VARIABILITY ON ESCHERICHIA COLI POPULATION DYNAMICS IN THREE DENVER, CO STREAMS

Waterborne illnesses continue to be a public health concern nationwide (Griffin et al. 2001). In the United States, the Environmental Protection Agency (EPA) along with local jurisdictions monitor and treat water used for drinking and recreational activity to prevent disease. Despite this vigilance, approximately 250,000 illnesses still occur every year from pathogenic contamination (Soller et al. 2010). An array of waterborne pathogens including *Cryptosporidium*, *Giardia*, *Norovirus*, *Salmonella*, *Escherichia coli*, *Legionella*, and *Hepatovirus* cause multiple adverse health effects including gastrointestinal illness, reproductive problems, and neurological disorders (Hlavsa et al. 2015). Populations especially susceptible to these illnesses include infants and young children, pregnant women, the elderly and immunocompromised patients (Hlavsa et al. 2015).

Testing for each waterborne pathogen is a time-consuming and costly endeavor. Thus, agencies use *E. coli* as a bacteriological indicator to test for fecal contamination, the likely source of most waterborne pathogenic diseases (EPA 2012). *E. coli* concentrations are strictly monitored to protect human health and to determine if water bodies are meeting federal and state regulatory compliance. However, *E. coli* populations are highly dynamic and are controlled by numerous abiotic factors at a variety of spatiotemporal scales such as UV exposure, turbidity, temperature and sediment entrainment. Consequently, relying on one static measurement for *E. coli* may not capture an accurate representation of *E. coli* concentrations, potentially resulting in

false positives which can result in federal and state non-compliance or false negatives which can risk public health exposure to disease. Given the complex interacting nature of abiotic factors on *E. coli* concentrations in ambient waters, a comprehensive field study that examines their joint influence is needed. This is of particular importance in the City and County of Denver where *E. coli* concentrations exceeded EPA standards in ten streams with only one meeting standards for recreational use in 2015. This study will significantly benefit Denver and its residents as the city continues to improve its water quality program and reach its goal of making all rivers and streams fishable and swimmable by 2020.

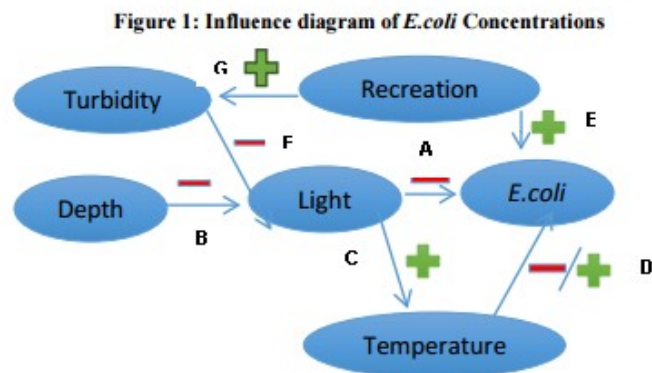
E. coli is a gram-negative, lactose-fermenting, coliform bacteria found in the intestinal tracts of warm-blooded mammals (Edberg et al. 2000). Most *E. coli* strains found in lakes, rivers and streams are not harmful but frequently co-occur with other pathogenic microbes that are passed into the environment by fecal matter (Blaustein et al. 2013). The strong correlation between fecal contamination and *E. coli* concentrations have made the bacteria a viable candidate for assessing water quality and public health risk (Griffin et al 2001). Although the bacteria have been used as a water quality indicator since the 1890's, quantitative measures of its concentration were unavailable at that time (Edberg et al. 2000). In the 1970's numerous studies found *E. coli* to be the only coliform inhabitant in warm-blooded gastrointestinal tracts, recognizing it as the best indicator for fecal contamination available (Edberg et al. 2000).

The birth of the Environmental Protection Agency (EPA) and the Clean Water Act (CWA) of 1972 paved the way for pathogen monitoring with the creation of the Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000. The BEACH Act directed the EPA to conduct studies that evaluated the relationship between pathogens and human health impacts (Recreational Water Quality Criteria 2012). Numerous comprehensive studies found a significant link between gastrointestinal illness and fecal contamination in water and the EPA revised the Recreation Water Quality Criteria in 2012 to better protect the public from high-

contact recreation water use (EPA 2012). The current standard set by the EPA for *E. coli*

concentrations in recreational waters is 126 colony-forming units (CFU: one viable cell capable of reproduction) per 100 milliliters of water (Sieuwerds et al. 2008). However, this standard is only a recommended level of protection, not a regulation, and it is the responsibility of states and local jurisdictions to adopt their own water quality criteria. Denver uses the EPA standard; even though many states use higher standards.

Even though strict standards now exist, *E. coli* populations vary both spatially and temporally as a result of several abiotic factors interacting, challenging compliance and increasing the risk to public health (Figure 1). One such challenge is that *E. coli* exhibits



significant temporal variation as a function of time of day and season (Desai et al. 2013). *E. coli* daily variation was measured over a 24-hour time scale in the San Jacinto River Basin to understand how often during the day a sample collection would lead to significantly different *E. coli* concentrations (Desai et al. 2013). *E. coli* was significantly lower during the afternoon compared to the morning, decaying at a rate of 3.67 to 24.7 CFU/day and bringing concentration levels below the EPA standard (Desai et al. 2013). However, the bacteria rapidly regenerated at night on the order of 9.41 to 64.1 CFU/day, returning concentrations back to pre-decay levels (Desai et al. 2013). After collecting samples at two different sites every three hours in the upper Hoosic River, Traister & Anisfeld (2006) also found higher *E. coli* concentrations in the early morning than in the afternoon as well as an accelerated decay rate throughout the day.

Solar radiation plays a pivotal role in *E. coli* concentrations and decay rates are largely dependent on light intensity (Rincón et al. 2004). This is because ultraviolet light inactivates

many forms of bacteria, including *E. coli* and is commonly used in laboratories for germicidal disinfection and the same effect is present in many streams, lakes and rivers (Figure 1, A) (Katara et al. 2008). Rincón et al. (2004) found that *E. coli* concentrations are predominately controlled by light intensity and that as sunlight intensity increased, the rate of decay increased as well. Furthermore, Fujioka et al. (2002) also found that the stability of *E. coli* and other viruses in the absence of sunlight were several orders of magnitude higher than the stability after exposure to one hour of summer and winter sunlight conditions (Fujioka et al. 2002).

Water temperature, a variable largely controlled by sunlight exposure, further influences *E. coli* population dynamics in complex ways (Figure 1, D). As temperature rises, UV inactivation rates gradually increase but so does the growth rate of *E. coli* up to a threshold temperature (Blaustein et al. 2004). Researchers determined the relationship of water temperature on *E. coli* concentrations using the Q_{10} equation, which can estimate the dependence of biological rates on temperature (Blaustein et al. 2004). Although results vary depending on the depth and size of the waterbody, *E. coli* did not decay at low temperatures (Blaustein et al. 2004). Consequently, the net direction and magnitude of the effect of temperature on *E. coli* concentrations varies both across and within watersheds. Many monitoring plans across the country emphasize *E. coli* concentrations in warmer seasons because the bacteria are thought to be less productive in the colder months. However, during the fall and spring bacterial concentrations may be more robust to a large range of temperatures.

These findings provide insight into an important component of water quality monitoring. The time of day a sample is collected has a significant impact on results. If municipalities like Denver collect samples in the morning, they might risk non-compliance, close recreational areas

and cause public concern because *E. coli* levels are overstated even though most recreational activity occurs more frequently in the afternoon. Additionally, collecting a sample once a day like most municipalities do, including Denver is not entirely representative and can significantly impact human health, possibly causing illnesses and public mistrust. Public health officials also need to be aware of cloud cover, shade, and depth when collecting samples because of the critical role that UV exposure plays in influencing *E. coli* concentrations. On cloudy days when there is less light and subsequently cooler water temperatures, *E. coli* concentrations will decay more slowly than on sunny days, resulting in higher concentrations. Samples taken in the shade will have a similar effect as well and Traister & Anisfeld (2006) attributed higher concentrations of *E. coli* at a site to the increase in shade compared to other sites in similar stream systems. This effect is present because the shade shields the water from solar radiation protecting *E. coli* from decaying as quickly as it would if solar radiation was greater. Additionally, rivers and streams are sampled 6 inches from the surface, per EPA protocol. However, solar radiation is highest at or near the water surface and the depth at which a sample is taken can also have a significant impact on results (Figure 1, B). Whiteman et al. (2004) found that *E. coli* concentrations at shallower depths in the morning exhibited more rapid decay over the course of a day compared to samples collected at greater depths. The complex dynamic between solar radiation intensity and time of day is important to consider when evaluating site locations for water quality assessments.

On the other hand, recreational activities like swimming and wading can increase *E. coli* concentrations and exposure risk when benthic sediments become suspended (Figure 1, E) (Alm et al 2003). Researchers evaluated the presence and concentration of *E. coli* in fresh, wet sand along six swimming beaches in Michigan (Alm et al. 2003). They found that at each beach, *E.*

coli counts in the sand were 3 -17 times higher than in the water (Alm et al. 2003). Because the bacteria adsorb to fine particulate sediments, sand acts as a reservoir for the bacteria (Brinkmeyer et al. 2015). Most urban freshwater swimming beaches contain sand and other fine sediments, some of which may harbor bacteria at depths greater than 60 cm. *E. coli* does particularly well in beach environments because it can use sand particles and other surfaces, such as algae, as a substrate where it can subsist outside of its hosts. Significant quantities of *E. coli* have been found on macroalgae in Lake Michigan (Ishii & Sadowsky 2008). Specifically, *Cladophora*, a common macroalgae found in rivers and lakes worldwide allows *E. coli* to survive for up to 6 months because leachate from algae provides nutrients to the bacteria (Byappanahalli et al. 2003). This cryptic supply of *E. coli* makes regulatory compliance and public health safety nearly unattainable (Brinkmeyer et al. 2015).

Recreational activity also increases turbidity in the water (Figure 1, G). Turbidity is an important factor for the survival of *E. coli* because high turbidity limits solar radiation throughout the water column, thus protecting *E. coli* inactivation (Figure 1, F) (Whitman et al. 2004). Furthermore, waters that are more turbid generally have cooler temperatures because the suspended particles shield the water from absorbing heat providing an adequate environment for the bacteria. Even though *E. coli* levels have a strong mid-day decay, sampling in an area that has any recreational activity can significantly increase *E. coli* concentrations via sediment resuspension and turbidity. It is important for water quality monitoring plans to be aware of recreation occurring upstream from sampling sites or sites that are in areas known for high recreational use because the relationship between sediment disruption and *E. coli* concentrations can pose a significant risk to public health and exceed the 126 CFU standard.

Many studies fail to examine the interactive nature of these influences. Research has investigated *E. coli* concentrations in the presence of abiotic factors, however the joint effect of multiple abiotic factors is under studied. Understanding how abiotic factors jointly influence *E. coli* populations throughout the course of a day can be challenging. It is imperative to gain a greater understanding of how each of these factors individually and in concert impact *E. coli* concentrations and their magnitude of change. This can significantly shape long-term monitoring plans and help jurisdictions achieve compliance nationwide. It can also significantly aid in our devoted protection to public health as we continue to discover the impact abiotic factors have on *E. coli* concentrations. The goal is to find a balance between reaching compliance and protecting public health in the midst of abiotic factors so Denver can reach its anticipated 2020 sustainability goal

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CHAPTER 2. GRANT PROPOSAL: INVESTIGATING THE INFLUENCE OF ABIOTIC VARIABILITY ON ESCHERICHIA COLI POPULATION DYNAMICS IN THREE DENVER, CO STREAMS

Abstract

Humans risk exposure to microbial pathogens when they use freshwater for recreation or as a drinking water source. In developing nations where monitoring and treatment of microbe-contaminated water is weak or unavailable, 1.8 million humans die each year from waterborne illnesses. In developed nations where public and private utilities treat drinking water, residents still remain vulnerable to these illnesses when they use recreational waters contaminated with sewage or animal feces. To assess whether streams and lakes should be closed to recreation, cities and counties closely monitor likely pathogen presence by testing for the indicator bacterium *Escherichia coli* (*E. coli*). Using *E. coli* concentrations to accurately portray a water body's contamination status remains a challenge because *E. coli* dynamics depend on multiple interacting factors including water depth, recreation intensity, light and temperature. To tease apart the relative importance of these factors on *E. coli* dynamics, I plan to measure *E. coli* concentrations as part of an observational field study in Denver, CO streams. Not only will this study provide a comprehensive portrait of citywide compliance with *E. coli* water quality standards, but it will also recommend improvements to sampling protocols. By lowering the false positive and false negative rate, these improvements will simultaneously help prevent waterborne illnesses and limit unnecessary closures of Denver's recreational waters.

Project Description

Background/Rationale/Significance

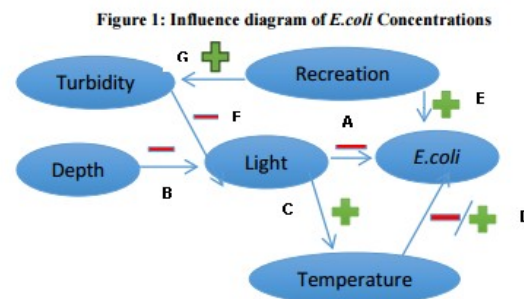
Waterborne illnesses continue to be a principal public health concern worldwide (Griffin et al. 2001). In developing nations pathogens that cause waterborne illness, frequently contaminate drinking water sources due to lack of policy and public works infrastructure. Consequently, 1.8 million people in these countries die from largely preventable illnesses each year (Ishii et al. 2008). Despite the widespread treatment of drinking water sources in the United States, contaminated water causes approximately 250,000 illnesses each year (Soller et al. 2010). Adverse health effects including gastrointestinal illness, reproductive issues and neurological disorders are caused by an array of waterborne pathogens including *Giardia*, *Norovirus*, *Salmonella*, *E. coli*, *Legionella*, and *Hepatitis A* (CDC 2014). Populations especially susceptible to these illnesses include infants and young children, pregnant women, the elderly and immunocompromised patients.

Pathogenic organisms contaminate recreational waters close to areas suffering from weak pipe infrastructure, lax local land-use practices and inefficient wastewater treatment. To minimize adverse health effects from these pathogen sources, jurisdictions monitor recreational streams and lakes for pathogenic organisms. Because testing

for each unique waterborne pathogen would be a time-consuming and costly endeavor, agencies monitor concentrations of the bacterium *E. coli* instead. Even though most *E. coli* strains are not pathogenic, it frequently co-

occurs with other pathogenic microbes (Blaustein et al. 2013). The current standard set by the EPA for *E. coli* concentrations in recreational waters is 126 colony-forming units (CFU: one viable cell capable of reproduction) per 100 milliliters of Water (Sieuwerts et al. 2008).

Watershed compliance with the EPA standard varies both spatially and temporally as a function of several interacting factors including depth, light exposure, temperature and recreational



activity (Figure 1). Exposure of surface waters to ultraviolet radiation present in sunlight inactivates *E. coli* and other bacteria. Thus, in un-shaded areas with higher light exposure *E. coli* concentrations decrease exponentially throughout the day and rebound at night, a phenomenon known as *diurnal sag* (Desai et al. 2013). Consequently, sampling in the morning results in higher *E. coli* concentrations that might overstate the true risk to residents because recreational activities typically take place later in the day. On the other hand, recreational activities like swimming and wading can increase *E. coli* concentrations and exposure risk when benthic sediments become suspended (Alm et al 2003). Water temperature, a variable largely controlled by light exposure, influences these dynamics in complex ways. As temperature rises, UV inactivation rates gradually increase but so does the growth rate of *E. coli* up to a threshold temperature (Blaustein et al. 2004). Consequently, the net direction and magnitude of the effect of temperature on *E. coli* concentrations varies both across and within watersheds. Because water samples for *E. coli* monitoring are typically taken from the water surface where water temperature and light are highest, true risk of exposure to *E. coli* at depth could be underestimated.

Given the complex interacting nature of the controls on *E. coli* concentrations in ambient waters, a comprehensive field study that examines their joint influence is needed (Blaustein et al. 2013). In the City and County of Denver, this is of particular importance because *E. coli* concentrations exceeded EPA standards in ten streams with only one meeting standards for recreational use in 2015 (Novick, 2015).

Clearly, understanding the interacting controls on *E. coli* dynamics would be important to accurately monitor pathogen loads in the area's recreational waters. A better understanding of these dynamics will likely help decrease false positives (i.e. closing a waterbody to recreation when human exposure risk is low) and false negatives (i.e. indicating a safe waterbody when human exposure risk is high). Consequently, I ask in this research study: **How does joint variation in UV**

exposure, temperature, depth and recreation intensity over the course of the day influence *E. coli* concentrations in Denver’s recreational use water bodies?

I will continue to work with the City and County of Denver’s Department of Environmental Health to better understand these dynamics. From this study we can recommend improved sampling techniques that portray a more accurate impact on human health, helping the city be compliant and achieve its goal of making all rivers and streams fishable and swimmable by 2020. This study aligns with a central mission at Regis University, a commitment to community service as well as a desire to educate others. *E. coli* concentrations may have a greater impact on lower income communities; this study can help serve such communities as we develop a greater understanding of population dynamics and how to protect those who are most at risk. Furthermore, this study will add to the ongoing research about *E. coli* ecology and its impact on human health.

Purpose and Specific Aims

The purpose of this study is to understand the relative strength and interactive nature of the proximal controls (e.g. recreational activity, depth, UV exposure and temperature) on spatiotemporal variation in *E. coli* population dynamics in Denver, Colorado streams. The results of this study will recommend improved sampling protocols to minimize human health risks. I aim to answer the following questions with this research study:

Specific Aims:

Q1. How does compliance with *E. coli* water quality standards vary as a function of UV exposure, temperature, depth and activity?

H1. I hypothesize that samples collected at depth in shaded streams with high recreational intensity will exceed *E. coli* water quality standards more frequently than samples collected at the surface in unshaded streams with low recreational intensity.

Q2. How does the *E. coli* decay rate vary as a function of UV exposure, temperature, depth and recreational activity?

H2. I hypothesize that the rate of *E. coli* decay will be significantly higher in surface samples in unshaded streams compared to samples at depth in shaded streams. Recreational activity can buffer this effect when absent, but exacerbate the effect when present.

Q3. How do abiotic factors jointly control *E. coli* concentrations?

H3. I hypothesize that Figure 1 represents the interactive nature of the controls of depth, temperature, light, and recreational activity on *E. coli* concentrations in Denver, CO streams.

Methods

Field Collection

In consultation with Jon Novick at the City and County of Denver, Department of Environmental Health, 20 sites stratified by recreational activity (10 high/10 low) and light intensity (10 shaded/10 unshaded) will be chosen from a database of 26 sites that are regularly sampled for *E. coli*. All sites have been categorized by DEH as either high use or not, based on historical recreation activity. Preliminary site visits will be conducted in early June 2017 to verify shade/light intensity by measuring light intensity with a light meter and canopy cover with a densiometer. Four sites will be randomly selected for sampling each week so that all 20 sites can be sampled in a five-week period (July 5th to August 9th). The random selection will ensure that each combination of recreational activity and light intensity will be represented during each sampling week. In a subsequent five-week period later in the season (September 6th to October 11th), the sampling scheme will be exactly replicated. The order of visits to the four sites will be randomly assigned each week, and the sampling will be conducted on a different weekday each week. Starting at 7:00 am on the chosen sampling day, water will be sampled for *E. coli* according to standard US EPA methods (EPA 2002). The water sample will be taken by hand at the water's

surface and at the deepest depth available at each site. The exact depth of sampling will be recorded because maximum depth will vary based on idiosyncrasies from site to site. Water temperature, conductivity, turbidity, dissolved oxygen and pH will also be recorded using a Horiba U52 probe. Light intensity will be measured with a light meter at the water surface.

Water samples will be immediately placed in a thermo-cooler stocked with ice so that the temperature remains below 10 °C. A thermometer will be closely monitored to ensure that samples remain within the temperature range and do not freeze. After sampling the first site, I will rotate to the next site until all sites have been sampled. Water at each site will then be sampled according to this rotation approximately every two hours until 5 pm, resulting in a total of 40 samples collected each sampling day. Samples will be immediately transported to the lab at Regis University where they will be held in a refrigerator at a constant temperature of no more than 10 °C and plated within 24 hours (EPA 2002)

Lab Analysis

Petri plates will be filled with M-TEC HiCrome™ (Sigma Aldrich) Agar, a chromogenic agar specially formulated to inhibit the growth of other bacteria (Giesser et al. 2000). The agar will be sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes, then cooled to 45-50°C and poured into sterile Petri plates.

To plate *E.coli* colonies, membrane filters with a (pore size of = .47 µm) will be used in conjunction with a funnel and vacuum system. The membrane will be placed in a filter funnel assembly connected to vacuum, which retains the bacteria. 100 ml of tenfold diluted stream water will be filtered through the filter assembly to achieve a plate that is countable. After filtration, the membrane will be transferred to a plate using sterile forceps. The petri dish containing M-TEC HiCrome™ Agar, a chromogenic agar specially formulated to inhibit the growth of other bacteria (Giesser et al. 2000). As per method 1603, Petri plates will be inverted and placed inside Whirl

Pak bags and incubated for 22-24 hours at 44.5°C. On M-TEC HiCrome™ Agar *E.coli* appears as a purple-magenta color visible to the human eye. Photos of each plate will be taken and analyzed with openCFU, a free software that allows colonies to be counted (opencfu.sourceforge.net). This method greatly increases time efficiency and accuracy (Giesser et al. 2000). CFU counts and all field data will be recorded onto data sheets for later statistical analysis. The funnel assembly will be decontaminated by removing the base from the funnel unit and using a germicidal ultraviolet (254 nm) light box to sanitize the equipment between filtrations. At least 2 minutes of exposure time is required. Googles will be used to protect the eyes from UV irradiation (Katara et al. 2008).

Statistical Analysis

Field and lab data from data sheets will be transferred to an Excel spreadsheet that will be used for data analysis in the statistical package R (R Core Team 2013). *E. coli* decay rates at each site will be calculated assuming first-order exponential decay kinetics (Brooks et al. 2016). The strength and direction of the relationship of the predictor variables temperature, light intensity, recreation intensity and depth (and their pairwise interaction terms) to the response variables *E. coli* concentration and decay rate will be evaluated using a multiple regression approach. The best regression model will be chosen using Akaike's Information Criteria (AIC), and significance of important predictors will be assessed using standard statistical hypothesis testing. Furthermore the interactive nature of the factors as they affect *E. coli* concentrations will be assessed from a structural approach. Specifically, I will assess my *a priori* hypothesis (Figure 1) of the interactive nature of the predictors and their influence on response variables with structural equation modeling, a statistical method used to examine simultaneous influences and responses in a single analysis (Grace 2006).

Work Plan

<u>Date</u>	<u>Activity</u>	<u>Deliverable</u>
June 2017	Preliminary sites visits to measure light intensity and canopy cover	Sites designated based on light intensity and canopy cover

July – August 2017	First phase of sampling for 20 sites and <i>E.coli</i> lab work	Data collected and organized for first phase of sampling
September 2017 – October 2017	Second Phase of sampling for 20 sites and <i>E.coli</i> lab work	Data collected and organized for second phase of sampling
October 2017-January 2018	Analyze and summarize all collected data	Data summaries and figures completed
February 2018	Complete Project Draft	Submit Draft to Supervisors for approval
April 2018	Complete final Project Draft	Present Study at URSC symposium

Relation to Course work/Career goals

I am currently an Environmental Biology graduate student with a strong interest in water quality. This research project will implement skills that I have acquired thus far in the program and will help further my education on water quality and environmental biology. I currently work for the Department of Environmental Health in Denver and plan to use my degree as well as my experience to continue working in the environmental health field.

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CHAPTER 3. JOURNAL MANUSCRIPT: INVESTIGATING THE INFLUENCE OF ABIOTIC VARIABILITY ON *ESCHERICHIA COLI* POPULATION DYNAMICS IN THREE DENVER, CO STREAMS

Abstract

To assess whether pathogen concentrations in streams and lakes pose a significant risk to human health, agencies monitor waterbodies by testing for the indicator bacterium *Escherichia coli* (*E. coli*). However, using *E. coli* concentrations to assess the presence of pathogens in waterbodies is challenging because *E. coli* dynamics depend on multiple abiotic factors (e.g., light exposure, temperature, recreational intensity) that interact across multiple spatiotemporal scales. To tease apart the relative importance of these factors on *E. coli* dynamics we collected water samples at 16 stream sites that varied in abiotic conditions in Denver, CO. At each site we measured *E. coli* concentrations every two hours over the course of the day to compare concentrations and decay rates at the surface and at depth. We found that 75% of all samples taken exceeded the EPA *E. coli* standard of 126 colony forming unit/100ml. While we found a significant difference between surface and bottom *E. coli* concentrations, only 25% of bottom concentrations were greater than surface concentrations. We also found no significant difference between surface and bottom decay rates. We used multiple regression models to investigate abiotic influence on concentrations and decay rates. Our results showed that *E. coli* concentrations were most negatively correlated with dissolved oxygen and turbidity and

positively correlated with specific conductivity. On the other hand, *E. coli* decay rates were most negatively correlated with pH and positively correlated with temperature. Although we expected decay rates to be stronger at the surface, our findings indicate that samples taken at surface or at depth have no predictable effect on concentrations and no effect on decay rates. Contrary to the findings of other studies in urban watersheds that show higher concentrations and weaker decay rates at greater depths, our results indicate that current *E. coli* sampling protocols for the City and County of Denver will accurately portray human health risk in streams.

Introduction

Waterborne illnesses continue to be a public health concern nationwide (Griffin et al. 2001). To prevent these diseases, the Environmental Protection Agency (EPA) and local jurisdictions monitor recreational water bodies by assessing *Escherichia coli* concentrations. *E. coli* has been used as a bacteriological water quality indicator for decades because its presence indicates fecal contamination, the likely source of most waterborne pathogenic diseases (Blaustein et al. 2013). However, aquatic *E. coli* populations are highly dynamic and are controlled by abiotic factors that interact at a range of spatiotemporal scales (e.g., ultraviolet light, turbidity, temperature, and sediment entrainment, Figure 1). Spatiotemporal variation in these drivers complicates accurate prediction of *E. coli* concentrations from one-time grab samples typically used by most municipalities.

E. coli concentrations exhibit significant diurnal fluctuation (over the course of a day) due to changes in sunlight which impact UV intensity and temperature (Ekklesia et al. 2015, Figure 1, A & B). Desai et al. (2013) measured *E. coli* concentrations over 24 hours in the San Jacinto River Basin and found that *E. coli* was significantly lower in the afternoon than the morning. The authors observed that typical decay rates brought concentration levels below the EPA standard of 126 (cfu) by the afternoon because ultraviolet light killed the bacteria. However, warmer water temperatures can instead increase bacterial growth until a threshold

temperature is reached (Blaustein et al. 2004). A positive correlation between *E. coli* concentrations and temperature was observed during summer months when stream temperatures averaged 20.4°C (Tiefenthaler et al. 2009) but when stream temperatures reach 25°C the lifespan of *E. coli* decreased resulting in high die-off rates (Guber et al. 2015).

In addition to those factors influenced by insolation, high sediment concentrations in the water column positively correlate with *E. coli* concentrations as well (Figure 1, C). *E. coli* concentrations measured in an urban watershed found that total suspended solid (TSS) concentrations more strongly correlated with *E. coli* concentrations than pH, temperature, specific conductivity, and nutrients (Wu et al. 2011). *E. coli* concentrations correlate positively with TSS because *E. coli* attaches to fine particles and accumulates in the benthic sediment (Muirhead et al. 2006). Swimming beaches in Michigan exhibited *E. coli* concentrations 3 - 17 times higher in the sediment than in the water column (Alm et al. 2013). Thus, sediments act as reservoirs for *E. coli* where it can multiply and survive for extended periods of time in the environment (Brinkmeyer et al. 2015).

In reality UV intensity, temperature, and turbidity do not act individually but rather interactively to influence *E. coli* concentrations over the course of a day. For example, water temperature is largely controlled by sunlight exposure and as temperatures increase from sunlight intensity, UV inactivation rates gradually increase as well (Figure 1, A, B, & C). The net direction and magnitude of the joint effect of temperature and sunlight varies spatially within a reach, and more broadly across reaches in the same watershed. For example, within a reach sunlight penetration and temperature decrease with increasing depth, thereby protecting *E. coli* populations below the surface from UV inactivation (Figure 1, F). Turbidity also limits the amount of solar radiation that penetrates throughout the water column protecting *E. coli* from sunlight induced inactivation (Figure 1, C). In warm water with high turbidity, concentrations can remain high during significant sunlight exposure because the increase in growth rate outpaces UV inactivation. On the other hand, high turbidity can signal recreation in the area or other

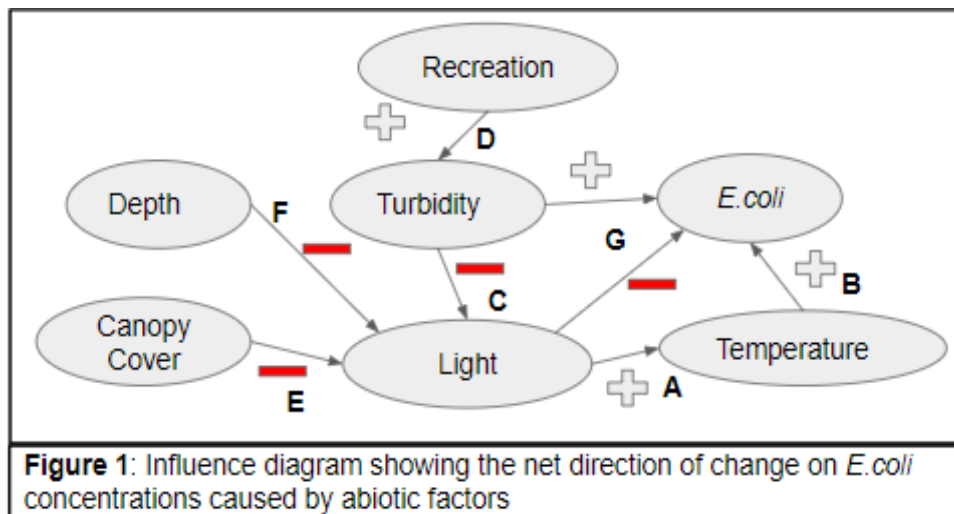
disturbances that release *E. coli* back into the water column from the sediment (Figure 1, D), consequently increasing *E. coli* concentrations.

Abiotic factors are also highly variable across reaches in the same watershed, further influencing *E. coli* dynamics. Traister and Anisfeld (2006) observed that slow-flowing and less shaded stream reaches have more pronounced diurnal fluctuations in *E. coli* density than on smaller, more shaded ones. Additionally, higher elevation streams within the same watershed exhibit smaller *E. coli* concentrations compared to lower elevation streams because higher elevations streams are less impacted by land-use practices and are more difficult for humans to access (Meays et al. 2006).

These interactions cause significant implications for current monitoring procedures. Currently, rivers and streams are sampled once a day and the time a sample is collected can have a significant impact on results. Typically, samples are taken six inches from the surface (EPA 2012) where solar radiation is the strongest, but concentrations at the surface may underestimate the *E. coli* concentration at depth. Other factors such as shade and cloud cover need to be evaluated because they block light and UV inactivation. Seasonality also influences concentrations because of seasonal variation in temperature, precipitation, and recreational activity in the water. A better understanding of these dynamics will likely help decrease false positives (i.e. closing a waterbody to recreation when human exposure risk is low) and false negatives (i.e. indicating a safe waterbody when human exposure risk is high). By understanding the abiotic drivers of *E. coli* dynamics, municipalities can better protect public health and limit the amount of beach closures that occur throughout the area.

To understand how joint spatiotemporal variation in abiotic controls drives changes in *E. coli* concentrations and its decay rates, we conducted a comprehensive field study in Denver,

CO. We assessed the relative strength, direction, and interactive nature of the proximal and ultimate controls on *E. coli* population dynamics using Figure 1 as our working hypothesis. If this hypothesis is correct, we predict that samples collected at depth in shaded streams with high recreational activity will have higher concentrations than samples collected at the surface in unshaded streams. Furthermore, we predict that the rate of decay will be significantly higher in surface samples collected in unshaded streams compared to samples collected at depth in shaded streams. The results of this study will provide a better understanding of the abiotic factors that control *E. coli* populations during the day in Denver. The City and County of Denver can implement improved sampling protocols that consider these relationships. In doing so, the city will continue to improve its water quality program and reach its goal of making all rivers and streams fishable and swimmable by 2020.



Methods

Study Area

Denver, Colorado (39.7392° N, 104.9903° W) is situated at the base of the Rocky

Mountains at an elevation of 1609 m. The area averages 245 sunny days per year and 43 cm of rainfall with most occurring between April – June. Many of Denver’s watersheds, including the South Platte River, Cherry Creek, Bear Creek, and their tributaries are heavily urbanized. Denver’s waterbodies are commonly used for recreation, and as such are routinely monitored by the Denver Department of Public Health and Environment (DDPHE) for the urban stressors of bacteria, nutrients, and heavy metals. In 2015, 15 out of 16 streams exceeded *E. coli* standards set by the US EPA for recreation.

High use recreation season in Denver generally runs from July- September and three waterbodies are commonly used for recreation are the South Platte River (SP), Cherry Creek (CC), and Bear Creek (BC). Residents use these waterbodies for swimming, kayaking, fishing, and pet recreation. We chose sites in each waterbody to assess citywide water quality compliance from a database of 40 sites that are routinely sampled throughout the year by DDPHE. We grouped sites according to waterbody and historic recreational use which was previously determined by DDPHE.

During preliminary site visits in June 2017, we assessed canopy cover and accessibility at each site. Sites that were difficult to access were eliminated and the remaining sites were categorized as shaded or unshaded. We narrowed our analysis to 16 sites, 4 in each watershed that represented a shaded site with high recreational use, a shaded site with low recreational use, an unshaded site with high recreational use, and an unshaded site with low recreational use (Figure 2).

Field Collection

We sampled water in each waterbody on separate collection days from July – September

(Figure 2). Starting at 8:00am, the most downstream site in each watershed was sampled

continuing upstream for the remainder of the sites. The rotation was repeated every two hours until approximately 4pm and we collected a total of 5 samples at each site. At every collection event, pH, temperature, conductivity, dissolved oxygen, and turbidity were measured using a Horiba U52 water quality probe. Light intensity was measured at the water surface using an Extech EA39 wide range light meter. Depth was measured using a meter stick to the nearest millimeter. Water samples were taken using 4 oz Whirl-Pak bags six inches from the surface of the water and again at the bottom. All samples were stored in a cooler with ice at 4°C and transported to the microbiology lab at Metropolitan State University every four hours to ensure the recommended holding time of 6 hours was not exceeded (EPA, 2012).

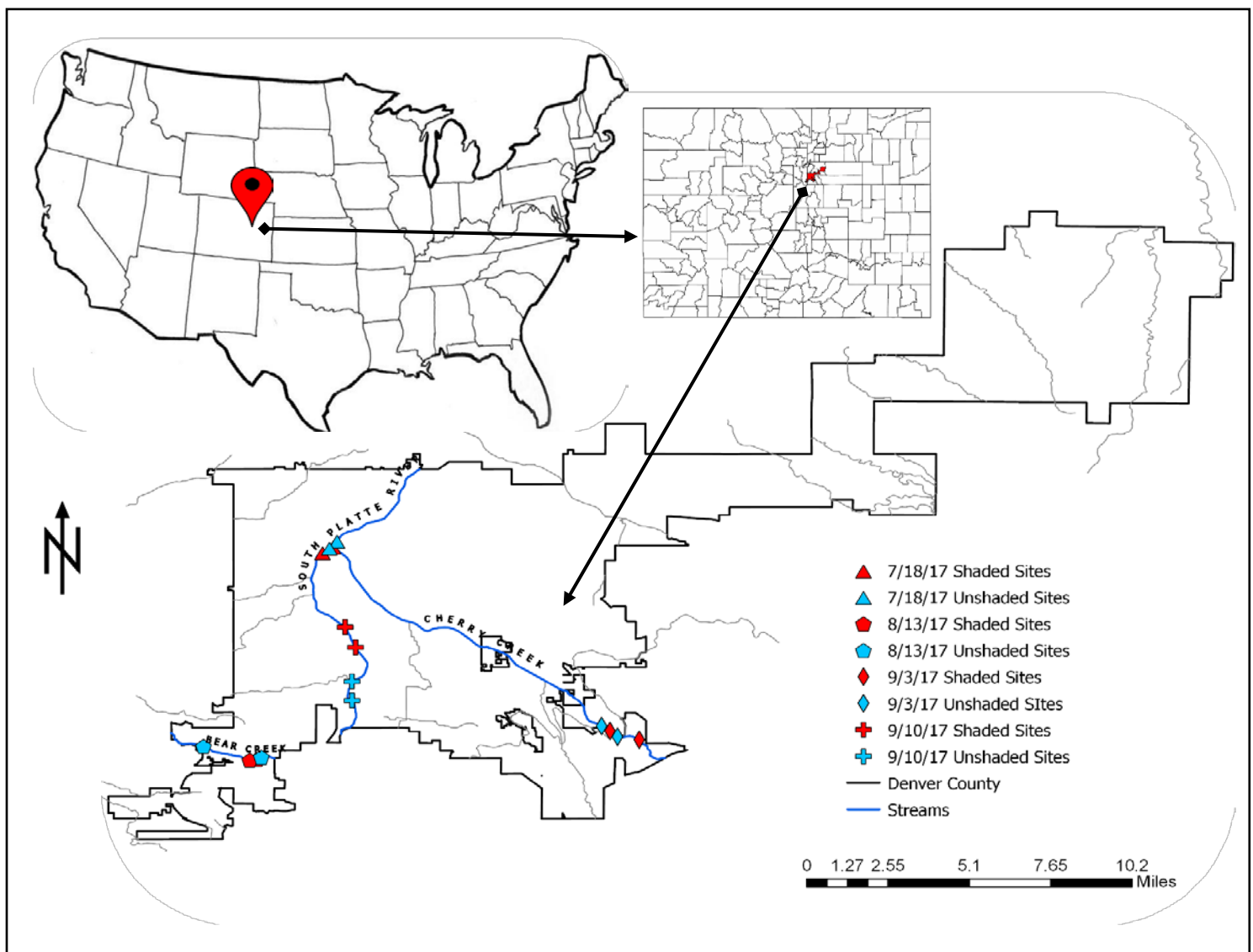


Figure 2: City and County of Denver watershed map with *E. coli* sampling locations at Bear Creek, South Platte River, and Cherry Creek are depicted

Laboratory Analysis

We estimated the most probable number (MPN) of *E. coli* in water samples using the IDEXX Quanti-Tray 2000/Colilert test method, which detects both coliform and *E. coli* in 24 hours (IDEXX, ME, USA). The contents of each Whirl-Pak bag were mixed with the Colilert reagent powder until dissolved and then poured into an individual Quanti-Tray. The tray was sealed using the Quanti-Tray Sealer Plus from IDEXX laboratories and immediately stored in an incubator at 37°C. After 24 hours of incubation, the tray was placed in an ultraviolet light black box and wells that fluoresced (positive for *E. coli*) were counted (Figure 3). A statistical method based on Poisson's law relates the abundance or MPN to the number of positive wells (Xue et al. 2018).



Statistical Analysis

To test the hypothesis that *E. coli* decay rates will be significantly higher in surface samples in unshaded sites compared to samples collected at greater depths in shaded streams, *E. coli* decay rates were calculated at each site at both surface and depth by assuming using first-order exponential decay kinetics (Brooks et al. 2016) in R (R Core Team 2013) with the following equation:

$$\ln[AA] = -k \cdot t + \ln[AA]_0$$

We developed a linear regression model for each site to predict log *E. coli* concentration as a function of time, depth, and their interaction. The slope of this regression model at each depth served as an estimate of the decay rate, *k*, while the intercept served as an estimate of the *E. coli* concentration at 8:00 am. We then developed contrasts using the `glht` function in the R

package multcomp (Hothorn et al. 2008) to compare both the rate of decay and morning *E. coli* concentrations at surface and at depth.

We then calculated the average value of each predictor variable over the course of the day (pH, temperature, specific conductivity, turbidity, sunlight intensity, dissolved oxygen). Using these averages, we assessed the individual strength and direction of relationship between our estimated decay rates and morning *E. coli* concentrations with each of those predictor variables as well as historical recreation use and shading status using a weighted linear regression. In these models, we weighted each point in the regression by the reciprocal of the squared standard error of the estimate. We then used the dredge function from the R package MuMIn (Barton 2018) to fit models of decay rate and *E. coli* concentrations as a function of all possible combinations of predictor variables and ranked them based on Akaike's Information Criteria (AIC) (Burnham & Anderson, 2004). A set of candidate models was defined using a threshold of $\Delta AIC \leq 7$. We then estimated the average coefficient for each variable if present in at least one of the candidate models (Fenberg et al. 2016).

Results

Study Site and Water Quality Characteristics

In total, we sampled 16 sites for *E. coli* and water chemistry over the course of a day. Samples were collected during dry weather flows and average daily precipitation throughout the study was .02 cm (NOAA, 2017). Abiotic variables across all sites and sampling events varied widely (Table 1). Unsurprisingly lux and canopy cover were negatively correlated such that for every 1% increase in canopy cover, light intensity decreased by 1.4 lux ($p = 9.8 \times 10^{-5}$,

$R^2=.6498$). In addition, we found that sunlight intensity in shaded ($Mean \pm SE: 18.18 \pm 2.56$) sites were significantly higher than unshaded ($Mean \pm SE: 52.97 \pm 3.31$) sites ($p = 1.96 \times 10^{-14}$). We also assessed the relationship between sunlight intensity and temperature and found that water temperature and lux were not significantly correlated ($p = 0.4281$, $R^2 = -0.001$). We then assessed the relationship between turbidity and temperature and found that for every 1°C increase in temperature, there was a .02 NTU increase in turbidity ($p = .02863$, $R^2 = .2481$, 95% CI: 0.002647622 to 0.04121208).

During our study South Platte River flows ranged from a minimum of 50 cfs to 1000 cfs and Cherry Creek flows ranged from 12 to 715 cfs (USGS, 2017). We could not obtain data for Bear Creek flows during our study. Our July 18th sampling date took place in both the South Platte River and Cherry Creek, and median daily discharge was 240 & 18 cfs (cubic feet per second) respectively (USGS, 2017). Median flow for September 3rd & 10th was 18 & 80 cfs, respectively for these streams (USGS, 2017).

Spatiotemporal drivers of variation in E. coli concentrations

75% of samples exceeded EPA standards of 126 cfu, regardless of depth, sunlight intensity, or recreational activity. Our estimated morning *E. coli* concentrations ranged from 1 - 2098 cfu at the surface and from 3.4 – 1553 cfu at depth across our 16 sites. 25.4% of our sites had higher concentrations at depth than at the surface, 37.2% had higher surface concentrations than at depth, and 37.6% were not significantly different ($p > .05$).

We also found that in univariate regression models, estimated morning *E. coli* concentrations did not significantly correlate with any measured abiotic variables (Table 2). However, when considering all possible models derived from our six abiotic variables, we found

that dissolved oxygen and turbidity negatively correlated with concentrations and specific conductivity positively correlated with concentrations ($R^2=.6294$). It was clear in all the best models that dissolved oxygen, turbidity, and specific conductivity are the most important factors influencing concentrations (Table 3). For every one mg/L increase in dissolved oxygen there was a 53 % decrease in median *E. coli* concentrations ($p = .000174$, 95% CI: 25 – 71%), for every e-fold increase in specific conductivity there was a three-fold increase in median *E. coli* concentrations ($p = 0.000242$, 95% CI: 2.1 to 6.2), and for every one NTU increase in turbidity there was a 1.5% decrease in median concentrations ($p = 0.024205$, 95% CI: -0.002 to 2.7%) (Table 3).

E. coli Decay Rate Spatiotemporal Relationships

At all 16 sites we found no significant difference ($p > 0.05$) between decay rates at the surface and at depth (Figure 4). Bottom decay rates ranged from -0.05 to 0.24 hr⁻¹ and surface decay rates ranged from -0.3 to 0.31 hr⁻¹. After confirming no significant difference between surface and bottom decay rates, we chose to use only surface decay rates to quantify their relationship with abiotic variables since municipalities sample from the surface.

In our univariate regression models, we found that surface decay rates were negatively influenced by pH ($p = .0255$, $R^2 = .259$) and positively influenced by specific conductivity ($p = .0304$, $R^2 = .2424$) (Figure 6, Figure 7, & Table 1, respectively). However, when considering all possible models, we found pH and temperature to be the most important factors influencing decay rates ($R^2 = .4391$). For every 0.1-unit increase in pH, median decay rates decreased by 6 (i.e. slower decay in more alkaline environments) ($p = .0069$, 95% CI: -1.9 to -9.9) and for every 1 °C increase in temperature, median decay rates increased by .08 (i.e. faster decay in warmer environments) ($p = .0356$, 95% CI: 0.0065 to 0.1599) (Table 3). The influence of pH on decay

rates in our study is strongly supported by our modeling results. Not only was the predictor included in all the best models, but it was also significant in each ($p < 0.05$).

Table 1: Abiotic variable data summary. Abiotic variables varied widely over the course of our study.

Variable	Mean	SD	Min	Median	Max
<i>Depth (cm)</i>	37.27	26.5	15.24	27.62	86.36
<i>Temperature (°C)</i>	20.98	2.33	15.51	21.25	25.96
<i>pH</i>	8.07	.1689	7.72	8.12	8.56
<i>Specific Conductivity (μS/cm)</i>	849.4	358.17	441	850.5	1660
<i>Turbidity (NTU)</i>	24.32	13.9	5	25.4	64.8
<i>Dissolved Oxygen (mg/L)</i>	8.73	.8284	7.27	8.69	11.67
<i>Sunlight Intensity (Lux)</i>	35.57	34.2	.4	21	137.7

Table 2: Results from simple univariate regression models predicting *E. coli* concentration and decay rate as a function of abiotic variables. “+” indicates a positive relationship and “-” indicates a negative relationship. Bolded *p*-values indicate $p < 0.05$. Morning concentrations had no significant relationship with any variable when modeled individually. Decay rate has a negative relationship with pH and a positive relationship and specific conductivity.

Predictor Variable	Relationship with <i>E. coli</i> Concentration	P-value (Concentration)	Relationship with <i>E. coli</i> Decay Rate	P-value (Decay)
Temperature	+	0.3287	+	0.1704
pH	-	0.3801	-	.02553
Specific Conductivity	+	0.0701	+	.0303
Turbidity	-	0.2121	+	.9827
Light Intensity	+	0.5169	+	.5959
Dissolved Oxygen	-	0.4605	+	.0560
Recreation	+	0.867	+	.2795

Table 3: Comparisons of candidate models estimating *E. coli* decay rate and *E. coli* concentrations as a function of abiotic variables across all sites. *E. coli* concentrations are most influenced by dissolved oxygen, turbidity, and specific conductivity and *E. coli* decay rates are most influenced by pH and temperature.

Predictors										
Model		Dissolved Oxygen	Specific Conductivity	Sunlight Intensity	pH	Temperature	Turbidity	AICc	Δ AIC	Weight
Concentration										
	4	-0.7557	1.2827				-0.0148	22.5797	0.0000	0.6310
	1	-0.7088	1.3732					25.2659	2.6862	0.1647
	2	-0.7256	1.1428			0.2150	-0.0178	25.8146	3.2349	0.1252
	3	-0.8481	1.1925		0.9111		-0.0192	26.7317	4.1520	0.0791
Model Average		-0.7595	1.2477		0.9111	0.2150	-0.0172			
Decay Rates										
	12				-0.5958	0.0832		-24.9660	0.0000	0.2913
	11				-0.5273			-22.9623	2.0037	0.1070
	10		0.0997		-0.3976			-22.9204	2.0456	0.1047
	9		0.1359					-22.6070	2.3590	0.0895
	8	0.0832			-0.4371			-22.5455	2.4205	0.0868
	7	0.0536			-0.5261	0.0691		-22.2070	2.7590	0.0733
	6				-0.6802		0.0024	-21.5954	3.3706	0.0540
	5	0.1101						-21.3829	3.5831	0.0486
	4		0.0400		-0.5297	0.0662		-21.0636	3.9024	0.0414
	3				-0.6502	0.0730	0.0010	-21.0140	3.9520	0.0404
	2			0.0005	-0.5586	0.0903		-20.8404	4.1256	0.0370
	1							-20.1387	4.8273	0.0261
Model Average		0.0823	0.0918	0.0005	-0.5447	0.0763	0.0017			

Notes: Bold face numbers are statistically significant regression coefficients ($P < 0.05$)

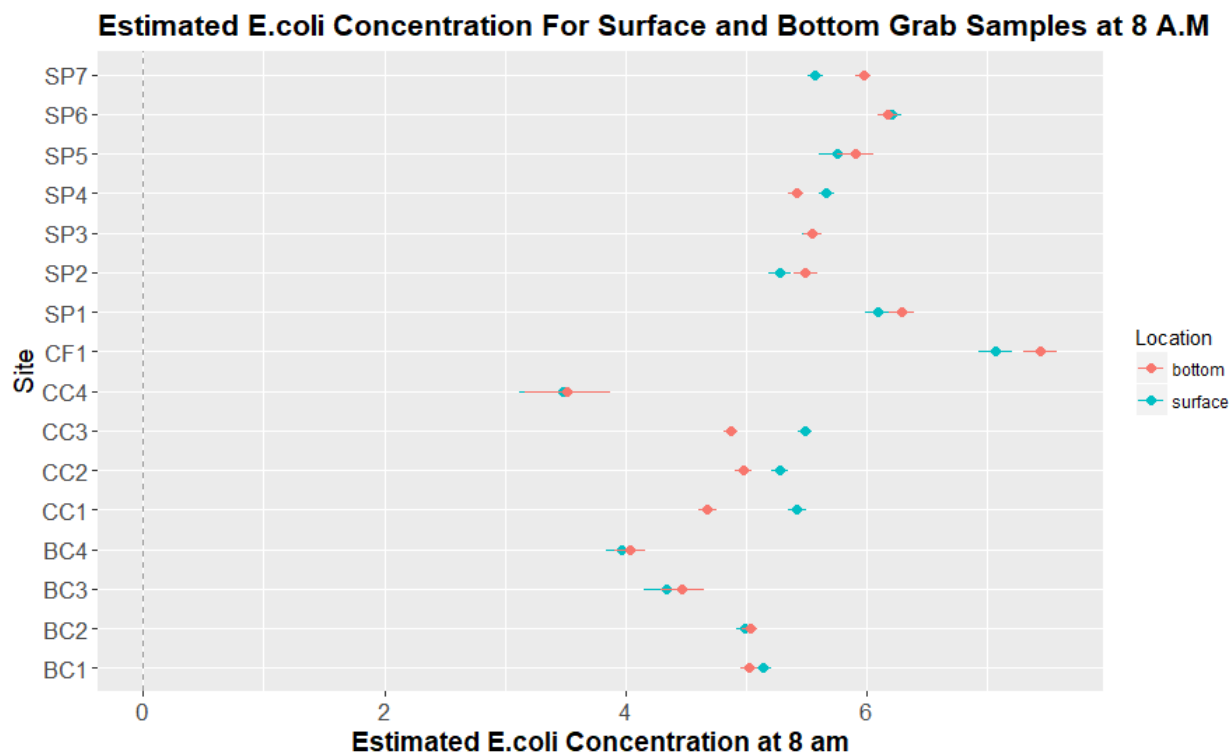


Figure 4: Surface and bottom concentrations significantly ($p < 0.05$) differed from each other in 62% of sampled sites. However, we did not observe a consistently higher concentration in bottom or surface as we had expected. Horizontal lines represent 2x the standard error and overlap indicates similarity in concentrations.

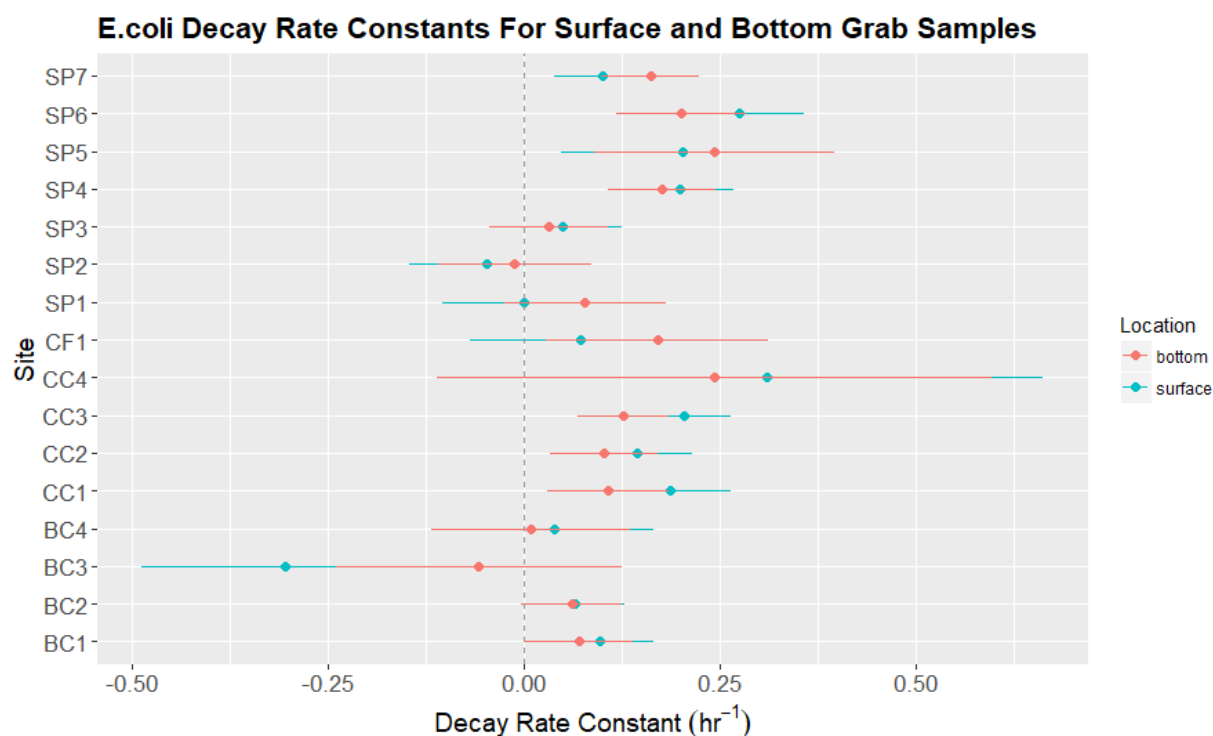


Figure 5: *E. coli* decay rates do not differ between surface and bottom grab samples. All p -values for all sites were >0.05 . Horizontal lines represent 2x the standard error. The large overlap of horizontal lines at each site show how similar surface and bottom decay rates are to each other. Daily decay rates were calculated for each site using first order exponential decay equation

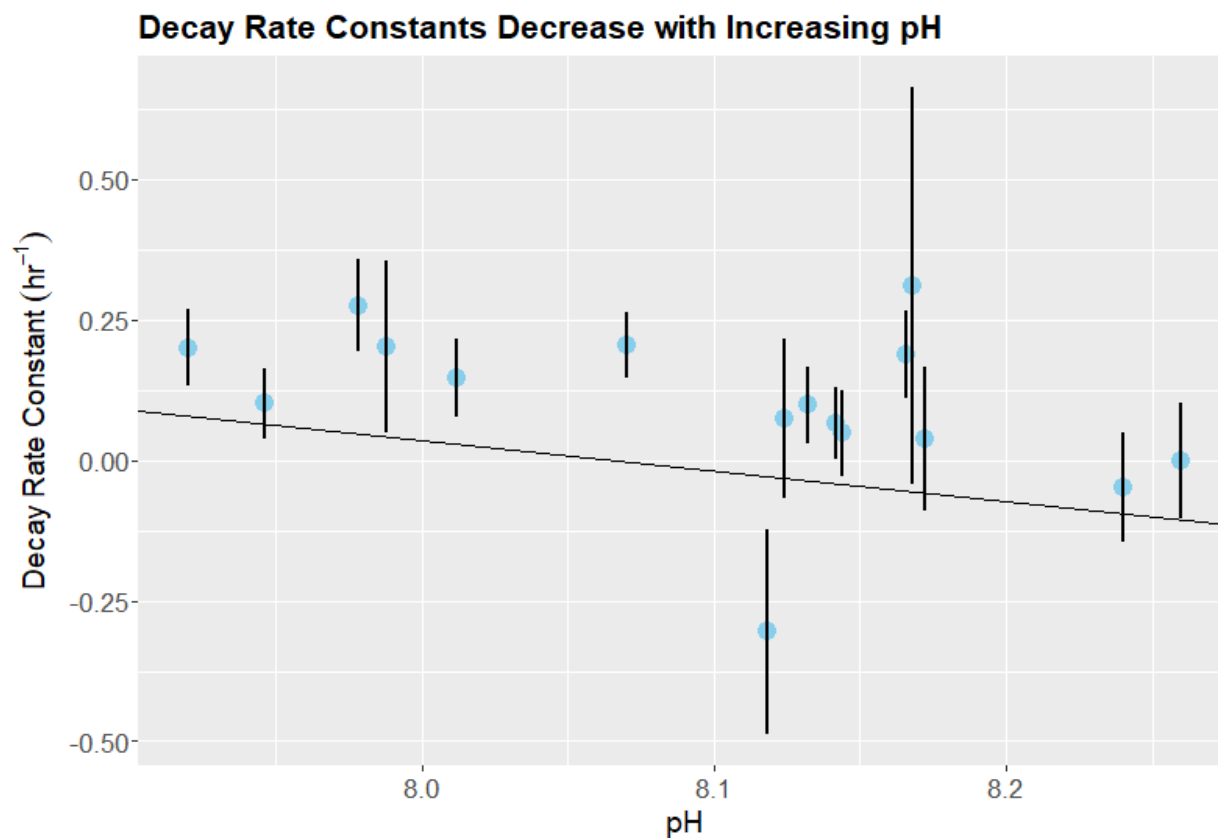


Figure 6: Decay rate constants decrease with increasing pH ($p = .02553$, $R^2 = .259$). Error bars represent 2x the standard error.

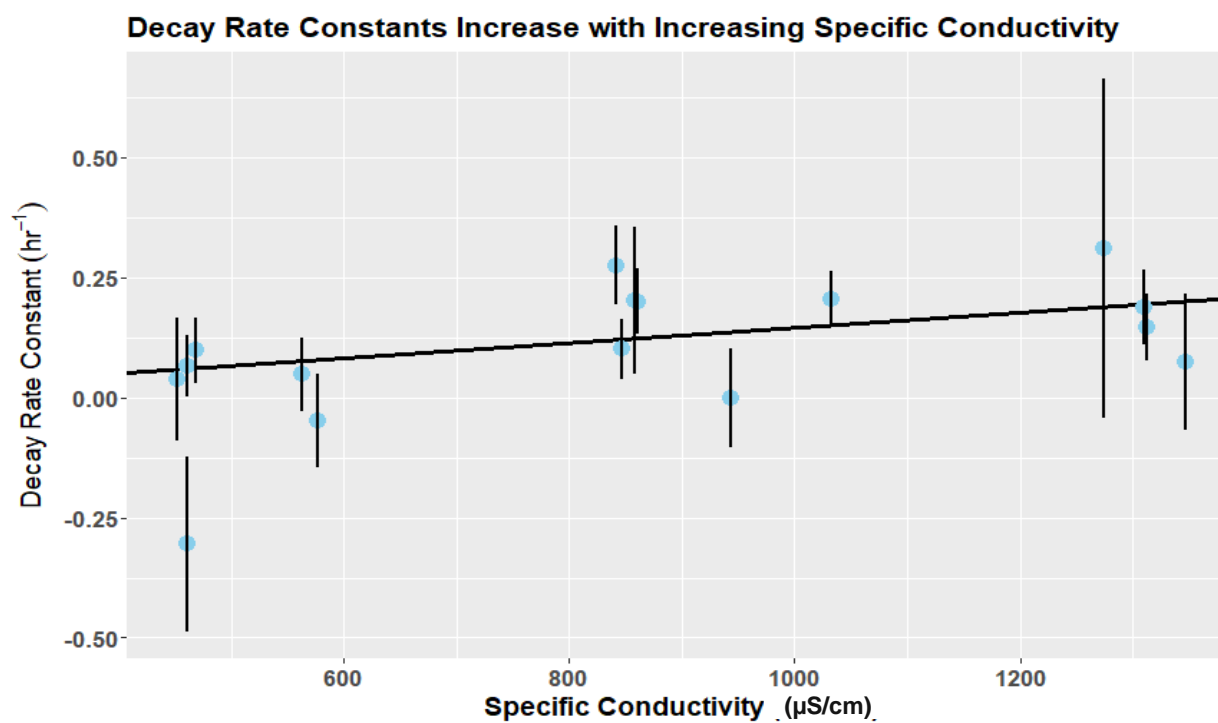


Figure 7: Decay rate constants increase with increasing conductivity ($p = 0.03039$, $R^2 = 0.2424$). Error bars represent 2x the

Discussion

Contrary to our predictions, our field study found no significant differences between surface and depth decay rates, regardless of shade intensity. We also found that decay rates were negatively correlated with pH and positively correlated with specific conductivity when modeled individually but are negatively correlated with pH and positively correlated by temperature when modeled together. Furthermore, we found that surface and bottom concentrations differed, but in no predictable direction. Although our sites varied in sunlight intensity, turbidity, and recreational use, none of these variables correlated with estimated concentrations or decay rates. Rather concentrations were strongly influenced by dissolved oxygen, turbidity, and specific conductivity when modeled jointly. Together, these results indicate that current *E. coli* sampling efforts by the City and County of Denver would not likely underestimate the *E. coli* concentrations in these waterbodies.

Although we found that surface and bottom concentrations differed, the direction of these differences followed no trend. Kleinheinz et al. (2006) also found that surface and depth concentrations significantly differed, however the authors observed that as depth increased, concentrations strongly decreased. We propose that the difference in our study and that of Kleinheinz et al. (2006) is due to their ability to sample across a wider range of depth (30 – 120cm) than we did. Our limited range could explain why we did not observe a more consistent pattern of bottom concentrations having greater *E. coli* densities than surface concentrations as we had predicted.

Dissolved oxygen, specific conductivity, and turbidity were the three most important factors influencing *E. coli* concentrations. The relationship between dissolved oxygen and

decreased concentrations found in our study is similar to other studies that have investigated the relationship between *E. coli* concentrations and dissolved oxygen (Rao et al. 2015, Kadir et al. 2004, Nevers & Whitman (2005), David & Haggard (2011)). Dissolved oxygen concentrations in aquatic environments is largely dictated by respiration from aquatic organisms, decomposition, and other chemical reactions. The presence of high dissolved oxygen in our study could simply indicate a low abundance of microorganisms and decomposition rates (O'Connor 1967). However, the relationship can also be a result of competition from indigenous bacteria in oxygen rich environments that inhibit *E. coli* growth, leading to a decline in *E. coli* concentrations (Wanjugi et al 2016).

Higher *E. coli* concentrations are often associated with higher turbidity levels and turbidity has been used as a surrogate to determine concentrations in past studies (David & Haggard 2011). However, our results show the contrary and we observed a decrease in concentrations with increasing turbidity. We propose our observed relationship is a result of a positive correlation between turbidity and temperature in this study. Higher turbid conditions have positively correlated with water temperatures in Kenya and turbid waterbodies were 2.8°C warmer than less turbid waterbodies (Paaijmans et al. 2009). Turbid conditions in our study could have likely insulated water temperatures leading to a die-off because of higher temperatures (Guber et al. 2015).

Higher specific conductivity is a signal for increased pollution in many streams (Fatoki et al. 2003) and could potentially be the driving factor behind the observed positive correlation between conductance and concentration. Little research has investigated this specific relationship. Due to the strength of the relationship we observed, further investigation should be completed in Denver to better understand the drivers in this relationship.

Each site in our study exhibited a daily decay for bottom and surface samples, however there was no observed difference in the rate of decay between surface and depth concentrations. Whitman et al. 2004 observed a pattern of stronger surface decay rates in their study largely due to sunlight inactivation at the surface. The authors concluded that the observed difference was largely attributed to a difference in turbidity between the depths of 45 and 90 cm. Turbidity in our study did vary, however turbidity was measured by placing the probe on the bottom of the stream. Gathering turbidity measurements at the surface and bottom in the future will allow us to make a more definitive determination as to the cause of our observed relationship.

According to our models, daily decay rate is negatively correlated with pH and positively correlated with temperature. *E. coli*'s ability to better survive in more alkaline environments has been observed in past studies and could explain this relationship (Jamieson et al. 2002). This relationship has implications for managing *E. coli* populations in urban waterbodies, especially Denver. A large amount of flow during dry weather events is the result of wastewater discharge upstream, which also have higher temperatures. The standard pH in Colorado wastewater discharges can be as high as 9 (Colorado Department of Public Health and Environment, 2017) which can aid in prolonging *E. coli* survival downstream. This can complicate current monitoring efforts and pose a risk to public health. Stronger decay rates observed in the presence of higher temperatures is likely due to the increased reaction rates at higher temperatures (Guber et al. 2015).

Our results, as well as other studies observed variability in *E. coli* concentrations throughout the day, challenging current sampling protocols established by US EPA. Current protocols recommend taking a one-time grab sample 15 cm from the water's surface to assess public health risk. However, studies conducted by Whitman et al. (2004) and Traister & Ansfeld

(2006) have shown that concentrations vary with depth and surface samples, and thus not entirely representative of concentrations throughout the waterbody. In 2010, the EPA released recommendations for site monitoring to be able to address these concerns (EPA, 2010). Along with considering site locations that have known fecal contamination and little variability, the EPA also recommends sampling in the morning to remain conservative, however many cities risk closing their water bodies upon the recommendation when recreational generally happen later in the day.

Even though our results support current sampling protocols established by the City and County of Denver, 75% of our samples collected exceeded the EPA standard of 126 cfu. This was to be expected because all three streams are listed on the 303d list for *E. coli* contamination (Colorado Department of Public Health and Environment, 2017). However, our findings further indicate that Denver, like other urban areas, has a chronic *E. coli* issue that can impact public health. Our sampling location at the confluence of Cherry Creek and South Platte contained the highest concentration of *E. coli* at any part of the day throughout all sampling events. After the summer of 2017, Denver began exploring this area as a potential source for an illicit discharge and is continuing to investigate the issue (Colorado Department of Public Health and Environment, 2017).

Our study encountered a few limitations that may have suppressed the strength of our predicted results. Overall, we expected sunlight to be one of the greatest driving factors in decay rate strength due to its ability to induce rapid mortality found in other studies like Desai et al. 2013 & Whitman et al. 2004 who determined that *E. coli* inactivation was largely affected by insolation. Surprisingly, *E. coli* decay rates were not stronger in unshaded sites and light intensity did not correlate with decay rates. Additionally, surface and depth samples should be

wider ranging in the future and exceed differences great than 25 cm. Flow also significantly varied between streams and we could have experienced a greater dilution effect of the bacteria on higher flow days or potentially a greater input of *E. coli* concentrations. Teasing apart the impact of flow on *E. coli* transport in Denver will help us better understand the dynamics we observed.

Although we did not find a strong relationship between decay rates and sunlight, we did find that pH and temperature have a strong control on *E. coli* population dynamics. Our findings can greatly benefit Denver's current monitoring program due to the improved understanding of the effect of abiotic factors on decay rates and concentrations. When waterbodies in Denver are experiencing higher pH values during instances such as wastewater discharges or seasonality, The City and County of Denver should increase monitoring to protect public health because of the influence of pH on decreasing decay rates. Denver should also consider monitoring during cooler water temperatures because cooler water temperatures may result in slower decay rates since we found that warmer water temperatures increase decay rates. Much debate has occurred in the water quality community around *E. coli* being a dependable indicator for public health risk due to the issue of variability. Unfortunately, aside from coliform bacteria, no other reliable sources of indicator bacteria have been used as a standard. In order to protect public health, we need to continue investigating the abiotic factors that control *E. coli* concentrations to improve sampling protocols for the protection of public health and improving water quality conditions.

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CHAPTER 4. ENVIRONMENTAL STAKEHOLDER ANALYSIS: A CALL TO BAN RIVERFRONT DEVELOPMENT IN DENVER, CO TO IMPORVE WATER QUALITY

Land use practices such as agricultural, residential, and commercial development have severely degraded water bodies worldwide. Such practices increase water pollution from both discrete point sources (i.e., pipes, wastewater effluent, factories) and diffuse non-point sources (i.e., roads, urban run-off), depositing heavy metals, chemicals, and bacteria into water bodies and decreasing water quality conditions (Tong & Chen, 2002). In the United States, water quality is governed by the Clean Water Act (CWA) to help protect the integrity of our water bodies as well as safeguard public health (Environmental Protection Agency, 2018). However, the number of impaired water bodies in urban areas is continuing to rise and many of them are threatened by *E. coli* contamination. Jurisdictions monitor for *E. coli* because its presence indicates fecal contamination, the likely source of most waterborne pathogenic diseases (Blaustein et al. 2013). In the City and County of Denver, 15 out of 16 streams exceeded *E. coli* EPA standards (City and County of Denver, 2017). With a rapidly growing population size and increased development in the Denver metro area, the issue is not going to improve without a significant change. To improve the integrity of Denver's water's and protect public health, I propose to institute a ban on riverfront development in the City and County of Denver starting in the year 2019 while we seek long terms goals for policy change and improved *E. coli* detection methods, while keeping stakeholder interest balanced.

Banning riverfront development would lessen any future adverse impacts to water quality in Denver while progress is made on policy and detection methods, however the ban would not be

without conflict. Developers in Denver have a large financial stake in construction throughout the city. Within the past 2 years, development has increased by 23% and \$7.8 billion in construction is underway or in the planning process (metrodenver.org, 2018). Since 2017, 11,056 new apartments have been added to the Denver market with an average renting price of \$1,350 (metrodenver.org, 2018). A substantial proportion of new construction is taking place along the waterfront because they are attractive, more expensive to rent, and in high demand. Additionally, the construction industry in Denver supports over 103,000 employees who depend on construction and development to support their own livelihood as well as their families (Denver Bureau of Labor Statistics, 2018).

Aside from developers, Denver residents are important stakeholders who have a strong affinity for Denver's waters. Residents use Denver water bodies for a multitude of activities including recreation and community gatherings. However, many residents risk contracting disease from pathogenic contamination and waterbodies can be closed to the public if the City does not meet EPA compliance. Common waterborne illnesses that occur from an array of pathogens include *Cryptosporidium*, *Giardia*, *Norovirus*, *Salmonella*, *Escherichia coli*, *Legionella*, and Hepatovirus. These pathogens can cause multiple adverse health effects including gastrointestinal illness, reproductive problems, and neurological disorders (Soller et al. 2010). Populations especially susceptible to these illnesses include infants and young children, pregnant women, the elderly and immunocompromised patients (Soller et al. 2010). Closing waterbodies due to public health risk is important for public safety but it is also frustrating for residents. Denver residents pay \$130 a year for capital projects that help maintain and improve Denver's recreational waterbodies (City and County of Denver, 2017). Residents are paying for accessibility and cleanliness and closing waterbodies because of contamination is financially unsettling. Not only do residents have a financial interest in the condition of Denver's rivers, streams, and lakes but many residents use Denver waterbodies for activities such as kayaking, fishing, and waterskiing. Denver has a large

population of Anglers who fish frequently on the South Platte River (Colorado Trout Unlimited, 2018). Accessibility is important for their sport and closing waterbodies due to public health risk would cause a large amount of frustration.

The public also depends on Denver's recreational waterbodies to provide spaces for community gatherings and entertainment. During the summer, Confluence Park, the point at which the South Platte River and Cherry Creek meet is filled with residents enjoying the view and swimming in the water. The City and County of Denver spent \$9 million dollars restoring the river bank and creating an open space for the public to enjoy (Kenny Andrew, 2017). However, this area is undergoing rapid residential development and has one of the highest *E. coli* concentrations in the city. Cherry Creek has also been listed on the Colorado Department of Public Health (CDPHE) impaired water's list for *E. coli* contamination (Colorado Department of Public Health and Environment, 2018). Confluence park is just one example among many of an area that brings people from multiple neighborhoods together to enjoy the amenities with their families, but also poses a significant public health risk.

Aside from the resident stakeholders, the city and county of Denver is concerned and invested in the water quality conditions and aesthetics of Denver's waterbodies. Denver Department of Public Health and Environment (DDPHE) routinely monitors Denver's rivers, lakes, and streams throughout the year to ensure that compliance is met (Denver Public Health and Environment, 2018). It is the responsibility of the City and County of Denver to safeguard public health and continue to work on improving water quality conditions. If they are unsuccessful, a large amount of public distrust can occur if residents are contracting waterborne diseases or access to swim beaches are closed. The City and County of Denver has a responsibility to their residents and visitors to ensure that public health is protected. The Denver Department of Public Health and Environment (DDPHE) samples waters in the city to assess conditions such as nutrient loads, heavy

metals and bacteria (Denver Public Health and Environment, 201). Even though no estimates have been found for illnesses caused by pathogens in Denver's recreational waterbodies, transmission likely occurs in Denver's waterbodies. The Center for Disease Control and Prevent (Center for Disease Control and Prevention, 2017) estimates that in 2017, nearly 7,000 deaths and 500,000 emergency room visits occurred due to 13 pathogens transmitted through urban waterbodies, including all pathogens that were previously listed. Public health risks will continue to rise in Denver as urbanization and population growth rise. By 2050, Denver is expected to double its population (denvergov.org). The rise in population will bring more residents and visitors to use Denver's waterbodies but the influx will ultimately increase water pollution. Growth in infrastructure, impervious surfaces, and sewage demand as well as more people in the water will pollute Denver waterbodies.

DDPHE works together with other non-profits in the area in engaging community members and reducing water pollution in Denver. One non-profit that is a significant stakeholder in the City is Groundwork Denver. The organization has done a large amount of work in helping improve Denver waterbodies and being a voice for the public (groundworkdenver.org). The non-profit acquired a grant from the Environmental Protection Agency and the Colorado Department of Public Health to conduct a study in Bear Creek, a popular stream for recreation that connects numerous low-income communities (GroundworkDenver.org). Bear Creek has exceeded *E. coli* levels for years and the source of the pollution is largely unknown (Denver Department of Public Health and Environment, 2018). With the work of Groundwork Denver and their partnership with DDPHE, they have been collecting samples along the creek. The goal is to be able to help determine the source of the pollution, protect community health, provide environmental education for inner-city students, and improve water quality conditions. Without the work of non-profits like

Groundwork Denver, protecting public health and improving the condition of Denver's waterbodies would be an even greater challenging task.

Even though strong partnerships exist between city agencies, residents and non-profits, water quality in Denver is severely impaired and development exacerbates the issue. Banning development on the waterfront can significantly limit the amount of pollutants that are directly flushed into the river system while the city works on long-term goals such as policy change and improved *E. coli* detection. The issue is complex and the proposal may not entirely satisfy developers because of the risk of losing valuable real estate, however if development continues and water sources are not protected developers risk losing sustainable clientele as residents leave the city to live elsewhere. Ultimately, the long-term goal is to protect public health and the integrity of Denver's waterbodies. While construction on the waterfront is halted, work can be done to institute stricter pollution regulations such as a reduction in permits issued for wastewater discharges and more stringent pollution concentration regulations. Additionally, there is no method for rapid *E. coli* detection and it takes 24 hours for results to be processed (EPA, 2004). During that lag time, residents are put at risk for pathogenic exposure. The EPA is working to achieve a method that would allow for near real-time determination of contamination, such that public notifications could be made sooner and cities could better protect public health. While these long-term goals are achievable, Denver need to limit any further impacts to water resources now. Banning waterfront construction is a solution that balances stakeholder interests from now and into the future.

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